Impact of Endophytic and Soil Microbes on Rice Grain Yield under Modern Bio-organo Mineral Fertilizer

R.A.G.H.L. SENEVIRATHNE[1], M. PREMARATHNA[2], B.L.W.K. BALASOORIYA[1] and G. SENEVIRATNE[2]

[1]*Department of Biotechnology, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP), 60170, Sri Lanka*

[2]*Microbial Biotechnology Unit, National Institute of Fundamental Studies, Hantana Rd, Kandy, 20000, Sri Lanka*

ABSTRACT

Excessive use of chemical fertilizers (CF) has reduced microbial abundances, causing degradation of agroecosystems with retarded soil-plant-microbial network interactions. Therefore, Biofilm Biofertilizer (BFBF) has emerged as a solution, replacing 50% of CF input. The modern Bio-organo Mineral (BOM) fertilizer is a novel practice of BFBF use, promoting fully organic agriculture. Since endophytic and soil microbes play a crucial role in plant growth and productivity, a field experiment was conducted across four districts (Ampara, Anuradhapura, Polonnaruwa, and Puttalam) during the 2023/2024 *Maha* season in Sri Lanka to examine their impact on rice grain yield under BOM fertilizer (BFBF + organic matter + natural minerals) and CF alone (Urea, TSP and MOP) with a control (no amendments) using 100 m^2 triplicate plots in RCBD design at each location. Endophytic microbial abundances were analysed from rice leaves, while soil microbial abundances were analysed from the root-zone soil and sub-soil (15-30 cm) of the treated plots by dilution plate method for bacteria, diazotrophs, and fungi. Results showed that, the average dry grain yield (GY) in the BOM fertilizer was 5874 kg/ha and that in CF was 5394 kg/ha. The BOM fertilizer practice recorded significantly higher endophytic microbial abundances (bacteria and diazotrophs) and soil microbial abundances (bacteria and fungi) than CF alone practice (Tukey's HSD test at $p<0.05$), contributing to the enhanced GY observed in the BOM fertilizer practice. Notably, abundances of endophytic microbes (bacteria and diazotrophs), and root-zone soil microbes (bacteria and diazotrophs) were close to the control, indicating that the BOM fertilizer maintains the natural microbial balance of the ecosystem, unlike CFs.

KEYWORDS: BOM fertilizer, Chemical fertilizer, Microbial abundances, Rice

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of the majority of the Sri Lankan population. Rice is cultivated mainly as a wetland crop in two seasons namely; *Maha* and *Yala* which are synonymous with the two monsoons.

Endophytic and soil microbes play a crucial role in plant growth and development. The endophytes endorse rice plant growth by producing phytohormones, solubilizing minerals, mitigating environmental adverse conditions, and protecting against phytopathogens by the production of secondary metabolites, lytic enzymes, antibiotics, and induced systemic acquired resistance, enhancing rice crop yield (Omomowo and Babalola, 2019). Furthermore, soil microbes enhance plant growth through aiding in resource acquisition (nitrogen, phosphorus, and essential minerals), regulating plant hormone levels, and mitigating pathogens through biocontrol activities (Gupta *et al*., 2000).

Although microbes play a significant role in rice cultivation, the microbial communities in rice fields exhibit considerable variability depending on the fertilizer application. Studies have shown that the application of organic fertilizers (OF) increases the abundance of soil (Subardja *et al*., 2016), and plant (Pariona-Llanos *et al*., 2010) biota compared to CF. Similar result for biofertilizers (BF) on soil biota (Arfarita *et al*., 2023) has been observed.

In the realm of sustainable agriculture, a type of BF, BFBF was introduced in the 2000s (Seneviratne *et al*., 2008). It has been reported that the application of BFBF can decrease the CF usage up to 50%, while increasing the yield by 20-30% and soil carbon sequestration by 30%, respectively (Premarathna *et al*., 2021; Jayasekara *et al*., 2022; Rathnathilaka *et al*., 2022). Moreover, BFBF restores degraded agroecosystems by enhancing soil-plant-microbial interactions (Premarathna *et al*., 2021). In addition, preliminary studies reported that the BFBF has a potential in fully organic agriculture (Navodya *et al*., 2023).

The BOM fertilizer, consisting of biofilm, organic matter and natural minerals is the novel trend of BFBF. However, this has not been tested in large-scale rice cultivation thus far. Therefore, the present study was designed to evaluate the effects of BOM fertilizer *vs*. CF on grain yield (GY) and microbial abundances in large-scale farmer-field trials.

METHODOLOGY

Study Area

The field experiments were carried out during 2023/2024 *Maha* season in four major rice-growing districts of Sri Lanka; Ampara (Dehiattakandiya), Anuradhapura (Thambuttegama), Polonnaruwa (Welikanda), and Puttalam (Arachchikattuwa). The study sites consisted of various climatic and soil conditions. Soil types vary from red-yellow podzolic with laterite, low humic gley to reddish brown earth (Ministry of Agriculture, 2014).

Field Experiments

In this study, four rice varieties commonly grown by the farmers were used *i.e*., Bg 403, Bw 367, Bg 360, and Bg 310 in Ampara, Anuradhapura, Polonnaruwa, and Puttalam districts, respectively. The treatments of the study were (a) BOM fertilizer [2.5 L/ ha of BFBF with 500 kg BOM/ ha (N- 275, P- 50 and K- 175 kg/ ha)], (b) CF alone [340 kg CF/ ha (Urea- 225, TSP- 55 and MOP- 60 kg/ ha)], and (c) control (no amendments). Each treatment was replicated three times, resulting in a total of separate nine plots at each site. Each plot was of size 10 m × 10 m with a good drainage system to prevent mixing of treatments. The treatment plots were arranged in a randomized complete block design at each site.

Sample Collection

At the 50% flowering stage, one randomly selected plant was carefully uprooted with root-zone soil from each plot by digging around the root zone without damaging the root system. Root-zone soil was sampled because it is the main sphere in which the root system explores essential resources for plant growth. Sub-soil (15-30 cm) samples were collected aseptically from each plot using a soil auger, carefully avoiding any cross-contamination between the sampling units (plots). The total sample number of root-zone soil and sub-soil was 72, and the plant number was 36 across the four districts. The collected samples were aseptically transported to the laboratory of the Microbial Biotechnology Unit, National Institute of Fundamental Studies (NIFS) at ambient temperature for further analysis.

Endophytic Microbial Analysis

The shoot endophytic total bacteria (ETB), endophytic diazotrophs (ED), and endophytic fungi (EF) were enumerated by dilution spread plate method, culturing them at 10^{-6} dilution in commercially available nutrient agar (NA) medium, combined carbon medium (CCM) (Rennie, 1981), and commercially available potato dextrose agar (PDA) medium, respectively. Media were prepared according to the composition and sterilized in an autoclave. The surfaces of the leaves were sterilized using 70% ethanol and distilled water series, followed by the extraction of endophytes (Sahu *et al*., 2022). The inoculated plates were incubated at the durations of 1-2 days, 3-4 days, and 5-6 days for bacteria, diazotrophs, and fungi, respectively. After the incubation period, the colony-forming units were counted and expressed as CFU mL^{-1}.

Soil Microbial Analysis

The root-zone soil total bacteria (RSTB), root-zone soil diazotrophs (RSD), root-zone soil fungi (RSF), sub-soil total bacteria (SSTB), sub-soil diazotrophs (SSD), and sub-soil fungi (SSF) were enumerated by dilution spread plate method, culturing them at 10^{-6} dilution. The soil samples were subjected to vigorous vortexing to facilitate the release of microbes adhering to soil particles prior to dilution series preparation (Zuberer, 1994). The NA medium, CCM, and PDA medium were used for bacteria, diazotrophs, and fungi, respectively. Media were prepared according to the composition and sterilized in an autoclave. The inoculated plates were incubated at the durations of 1-2 days, 3-4 days, and 5-6 days for bacteria, diazotrophs, and fungi, respectively. After the incubation period, the colony-forming units were counted and expressed as CFU mL^{-1}.

Grain Yield Analysis

At crop maturity, 5 m × 5 m crop cuts were utilized non-randomly within each plot. The dry grain weights from these crop cuts were measured and the GY was calculated in kg/ha.

Statistical Analysis

Analysis of Variance (ANOVA) was applied to determine the treatment effects. As a post hoc analysis, Tukey's HSD test was performed for comparison of treatment means. Simple correlation was performed to test the relationships among the variables. The probability level considered for the statistical significance of the results was 0.05, and all the data were analysed using Minitab 19 version.

RESULTS AND DISCUSSION

Average Grain Yield

There were no significant differences observed between the two fertilizer practices for average GY over four study sites (p = 0.524, Table 1), but the average grain yield in the BOM fertilizer was 5874 kg/ha and that in CF was 5394 kg/ha (Table 1). The Department of Agriculture (DOA) has determined applying 10 tons/ha of OF along with 70% CF will yield the same result as 100% CF application (Weerasinghe, 2023). In this study only 500 kg/ha of OF was used, achieving a comparable yield despite lower OF application.

Effect of Endophytic Microbes on Grain Yield

The results indicated that two fertilizer applications have significantly affected on the entophytic microbial abundances. Specifically, the abundances of ETB, and ED were significantly higher in the BOM fertilizer practice compared to the CF alone practice. Notably, there was no any significant difference in EF abundance between the BOM fertilizer practice and the CF alone practice, but the abundances were higher in these two practices than the control (p<0.05, Table 1).

Previous studies also showed higher ED abundances in BFBF practice (Premarathna *et al*., 2021) and in OF practice (Pariona-Llanos *et al*., 2010). Similar results for ETB have been found with OF practice (Wang *et al*., 2022).

The high abundances of ETB and ED in BOM fertilizer practice could have a positive effect on grain yield because the ETB mitigates plant stress and enhances growth by biological N_2 fixation, phosphate solubilization, siderophore production, and synthesis of growth promoting substances (Prasad *et al*., 2020), and ED enhances plant growth by nitrogen fixation, hormone production, nutrient uptake improvement, pathogen suppression, phosphate solubilization, and increases stress tolerance (Carvalho *et al*., 2014).

Moreover, the ETB showed a significant positive correlation with GY (r = 0.624, p = 0.030, Figure 1 A) in the CF alone practice, indicating that the ETB was limiting in the system with its lower abundance compared to the BOM fertilizer practice (Table 1). By adding BOM fertilizers, the ETB could be optimized to achieve increased GY. In the BOM fertilizer practice, the result of the present study on EF may be due to the competition of ETB with EF, for the limited colonization space and nutrients provided by the rice plant (Mano and Morisaki, 2008).

Notably, the microbial abundances (bacteria and diazotrophs) in the BOM fertilizer practice were lower than that in the control (Table 1). However, the values were closer to the control than the values observed in CF practice. This may be due to the effect of BFBF on breaking the dormancy of microbial seed banks, preserving natural ecosystem integrity (Seneviratne and Kulasooriya, 2013).

Effect of Soil Microbes on Grain Yield

The results indicated that two fertilizer applications have significantly affected on the microbial abundances in both root-zone soil and sub-soil. Specifically, the abundances of RSTB, RSF, SSTB, and SSF were significantly higher in the BOM fertilizer practice compared to the CF alone practice, while the SSD abundance was significantly higher in the CF alone practice than the BOM fertilizer practice. Notably, the RSD abundance in the BOM fertilizer practice was not significantly different from the other two practices (p<0.05, Table 1).

Previous researches also have shown higher soil bacterial and fungal richness in BFBF practice (Rathnathilaka *et al*., 2022), and in OF practice (Chang *et al*., 2007) while, CF contribute to the decreasing of the richness of these microbes. Furthermore, mineral fertilizers (N, NP, NPK) combined with organic matter are effective for increasing the quantity of soil microbes (Gu *et al*., 2008).

A similar study was conducted by Nakhro and Dkhar (2010) which compared the use of OF with chemical ones. Deviating from the results of the aforementioned study, the present study reported a higher abundance of bacteria in the root-zone soil in both BOM fertilizer practice and control (Table 1). This could be attributed to the greater sensitivity of soil

Table 1. Average yield and microbial abundances under two fertilizer practices in rice cultivation

Parameter	Fertilizer practice			
	BOM fertilizer	**CF alone**	**Control**	***P* – value**
ETB (× 10^7 CFU mL^{-1})	$33.8^b \pm 0.8$	$25.2^c \pm 0.9$	$37.3^a \pm 0.8$	0.000
ED (× 10^7 CFU mL^{-1})	$13.6^b \pm 0.7$	$3.1^c \pm 0.8$	$25.8^a \pm 0.6$	0.000
EF (× 10^7 CFU mL^{-1})	$14.6^a \pm 2.1$	$12.4^a \pm 0.9$	$7.2^b \pm 1.1$	0.004
RSTB (× 10^7 CFU mL^{-1})	$68.7^a \pm 3.7$	$45.7^b \pm 1.7$	$80.9^a \pm 6.7$	0.000
RSD (× 10^7 CFU mL^{-1})	$17.6^{ab} \pm 2.3$	$14.9^b \pm 2.6$	$24.8^a \pm 2.2$	0.017
RSF (× 10^7 CFU mL^{-1})	$6.8^a \pm 1.9$	$1.6^b \pm 0.5$	$1.2^b \pm 0.3$	0.002
SSTB (× 10^7 CFU mL^{-1})	$56.4^a \pm 1.9$	$44.1^b \pm 3.6$	$22.2^c \pm 1.9$	0.000
SSD (× 10^7 CFU mL^{-1})	$13.2^b \pm 1.3$	$21.3^a \pm 1.3$	$8.4^c \pm 1.1$	0.000
SSF (× 10^7 CFU mL^{-1})	$4.5^a \pm 0.8$	$0.7^b \pm 0.3$	$0.4^b \pm 0.2$	0.000
GY (kg/ha)	$5874^a \pm 450$	$5394^a \pm 533$	$5034^a \pm 557$	0.524

Mean ± SE in each column. Within rows, values with different letters differ significantly (p<0.05, Tukey's HSD test). ETB- Endophytic total bacteria; ED- Endophytic diazotrophs; EF- Endophytic fungi; RSTB- Root-zone soil total bacteria; RSD- Root-zone soil diazotrophs; RSF- Root-zone soil fungi; SSTB- Sub-soil total bacteria; SSD- Sub-soil diazotrophs; SSF- Sub-soil fungi; CFU- Colony forming unit; GY- Grain yield; BOM- Modern bio-organo mineral and Cf- Chemical fertilizer

(A)

	ETB	ED	EF	RSTB	RSD	RSF	SSTB	SSD	SSF
ED	-0.041 (0.905)								
EF	0.741 (0.006)	0.536 (0.089)							
RSTB	0.021 (0.951)	0.109 (0.764)	0.007 (0.985)						
RSD	-0.416 (0.204)	-0.110 (0.762)	-0.676 (0.022)	0.617 (0.043)					
RSF	-0.549 (0.064)	0.026 (0.940)	-0.129 (0.690)	-0.171 (0.616)	-0.285 (0.395)				
SSTB	0.601 (0.039)	0.056 (0.869)	0.442 (0.150)	0.726 (0.011)	0.528 (0.095)	-0.571 (0.053)			
SSD	0.189 (0.578)	0.212 (0.532)	0.186 (0.585)	0.853 (0.002)	0.611 (0.060)	-0.180 (0.597)	0.520 (0.101)		
SSF	-0.345 (0.272)	0.010 (0.978)	-0.161 (0.618)	-0.147 (0.666)	-0.256 (0.448)	0.510 (0.090)	-0.485 (0.110)	0.171 (0.616)	
GY	0.624 (0.030)	-0.373 (0.259)	0.216 (0.499)	-0.278 (0.408)	-0.120 (0.726)	-0.413 (0.182)	0.393 (0.206)	-0.158 (0.643)	-0.189 (0.557)

(B)

	ETB	ED	EF	RSTB	RSD	RSF	SSTB	SSD	SSF
ED	0.988 (0.000)								
EF	0.341 (0.305)	0.399 (0.199)							
RSTB	0.090 (0.805)	0.084 (0.807)	0.027 (0.936)						
RSD	0.405 (0.216)	0.368 (0.239)	-0.401 (0.197)	0.528 (0.095)					
RSF	-0.061 (0.858)	-0.141 (0.662)	-0.540 (0.070)	0.004 (0.991)	0.208 (0.517)				
SSTB	-0.083 (0.819)	-0.224 (0.507)	-0.450 (0.165)	0.189 (0.601)	0.290 (0.386)	0.364 (0.272)			
SSD	0.065 (0.849)	-0.053 (0.871)	-0.333 (0.290)	0.267 (0.427)	0.293 (0.355)	0.284 (0.371)	0.954 (0.000)		
SSF	0.264 (0.433)	0.117 (0.718)	-0.323 (0.306)	-0.018 (0.959)	0.372 (0.234)	0.506 (0.094)	0.591 (0.055)	0.492 (0.104)	
GY	-0.127 (0.710)	-0.020 (0.952)	0.245 (0.443)	-0.121 (0.722)	-0.445 (0.148)	-0.186 (0.562)	-0.771 (0.005)	-0.713 (0.009)	-0.469 (0.124)

Figure 1. Pair-wise correlation matrices between average yield and microbial abundances in rice cultivation under two fertilizer practices

(A) CF alone; (B) BOM fertilizer. Pearson correlation in each column. Values within parentheses are p – values. Values in highlighted cells are significant at p<0.05 ED- Endophytic diazotrophs; EF- Endophytic fungi; RSTB- Root-zone soil total bacteria; RSD- Root-zone soil diazotrophs; RSF- Root-zone soil fungi; SSTB- Sub-soil total bacteria; SSD- Sub-soil diazotrophs; SSF- Sub-soil fungi; GY- Grain yield;

bacteria than fungi to added fertilizers (Benizri and Amiaud, 2005).

The high abundance of both bacteria and fungi in BOM fertilizer practice could have a positive effect on grain yield, since they enhance plant growth directly by aiding in resource acquisition (nitrogen, phosphorus, and essential minerals), regulating plant hormone levels, and indirectly mitigating the inhibitory impacts of diverse pathogens through biocontrol activities (Gupta *et al*., 2000).

Although there was no significant difference between the two fertilizer practices on RSD, an average value of 17.6×10^7 CFU mL^{-1} was observed in the BOM fertilizer practice compared to that of 14.9×10^7 CFU mL^{-1} in CF practice (Table 1).

The reason for the lower SSD abundance in the BOM fertilizer practice than in CF practice could be the enriched N levels in the sub-soil due to the high organic matter application (Edmeades, 2003), resulting lower diazotrophic community (Wang *et al*., 2022). Moreover, the SSD showed a significant negative correlation with GY (r = -0.713, p = 0.009, Figure 1 B), indicating lower SSD abundances enhance the GY in BOM practice.

Notably, the microbial abundances in root-zone soil (bacteria and diazotrophs) under the BOM fertilizer practice were close to the control (Table 1), indicating that the BFBF helps to activate microbial seed banks, promoting biodiversity and preserving natural ecosystem integrity (Seneviratne and Kulasooriya, 2013).

Furthermore, the absence of significant correlations between the abundances of microorganisms except ETB and ED, and SSTB and SSD observed in the BOM fertilizer practice could be attributed to the higher microbial abundances in both plants and soil after the application of BOM fertilizer.

CONCLUSIONS

The BOM fertilizer positively affects GY despite lower organic fertilizer application, and results in higher abundances of endophytic microbes (bacteria and diazotrophs), and soil microbes (bacteria and fungi) than CF practice, contributing to the enhanced GY. Notably, abundances of endophytic and root-zone soil microbes (bacteria and diazotrophs) were close to the control, highlighting its potential as a sustainable and effective alternative to CFs.

ACKNOWLEDGEMENTS

The authors sincerely thank the staff of the Microbial Biotechnology Unit, NIFS, Department of Biotechnology, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, and the collaborated farmers. This research was conducted and fully funded by the NIFS.

REFERENCES

Arfarita, N., Imai, T. and Prayogo, C. (2023). Effect of inorganic fertilizer and VP3 biofertilizer applications in legume on the population of indigenous bacteria. *Journal of Agricultural Science*, **45**(3), 467-482.

Benizri, E. and Amiaud, B. (2005). Relationship between plants and soil microbial communities in fertilized grasslands. *Soil Biology and Biochemistry*, **37**, 2055-2064.

Chang, E. H., Chung, R. S. and Tsai, Y. H. (2007). Effect of different application rates of organic fertilizer on soil enzyme activity and microbial population. *Soil Science and Plant Nutrition*, **53**(2), 132-140.

Carvalho, T.L.G., Balsemão-Pires, E., Saraiva, R.M., Ferreira P.C.G. and Hemerly, A.S. (2014). Nitrogen signaling in plant interactions with associative and endophytic diazotrophic bacteria. *Journal of Experimental Botany*, **65**(19), 5631-5642.

Edmeades, D. C. (2023). The long-term effects of manures and fertilisers on soil productivity and quality. *Nutrient Cycle Agroecosystems*, **66**, 165-180.

Gu, Y., Yun, X., Zhang, X., Tu, S., Xi, F., Sun, X. and Lindström, K. (2008). Effect of different fertilizer treatments on quantity of soil microbes and structure of ammonium oxidizing bacterial community in a calcareous purple rice soil. *Agricultural Sciences in China*, **7**(12), 1481-1489.

Gupta, A., Gopal, M. and Tilak, K. V. B. R. (2000). Mechanism of plant growth promotion by rhizobacteria. *Indian journal of Experimental Biology*, **38**, 856-862.

Jayasekara, A., Ekanayake, S., Premarathna, M., Warnakulasooriya, D., Abeysinghe, C. and Seneviratne, G. (2022). Organic material inputs are not essential for rice soil carbon sequestration. *Environmental Challenges*, **8**, 100551.

Mano, H. and Morrisaki, H. (2008). Endophytic bacteria in the rice plant. *Microbes and Environments*, **23**(2), 109-117.

Ministry of Agriculture (2014). Soil Groups of Sri Lanka [Accessed on 22.05.2024] Available at <http://www.agrimin.gov.lk/web/index.php/en/downloads/maps>.

Nakhro, N. and Dkhar, M. S. (2010). Impact of organic and inorganic fertilizers on microbial populations and biomass carbon in rice field soil. *Journal of Agronomy*, **9**(3), 102-110.

Navodya, M.H., Premarathna, M., Seneviratne, G., Gunasekara, N.W. and Herath, S. (2023). Potential use of biofilm biofertilizers in fully organic rice cultivation of Sri Lanka. Proceedings of the Seventh International Research Conference, Uva Wellassa University, Badulla, pp. 298.

Omomowo O. I. and Babalola, O. O. (2019). Bacterial and fungal endophytes. *Microorganisms*, **7**, 481.

Pariona-Llanos, R., Ferrara, F. I. S., Gonzales, H. H. S. and Barbosa, H. R. (2010). Influence of organic fertilization on the number of culturable diazotrophic endophytic bacteria isolated from sugarcane. *European Journal of Soil Biology*, **46**(6), 387-393.

Prasad, M., Srinivaasan, R., Chaudhary, M., Mahawer, S. K. and Jat, L. K. (2020). Endophytic bacteria, In: Ajay Kumar, A., Singh. K. V. (Eds.), Food Science, Technology and Nutrition, Woodhead Publishing Inc., 2020, pp 37-60.

Premarathna, M., Seneviratne, G., Ketipearachchi, K. G., Pathirana, A., Karunaratne, R. K. C., Balasooriya, W. K. and Fonseka, K. (2021). Biofilm biofertilizer can reinstate network interactions for improved rice production, *Ceylon Journal of Science*, **50**(3), 235-242.

Rathnathilaka, T., Premarathna, M., Madawala, S., Pathirana, A., Karunaratne, K. and Seneviratne, G. (2022). Biofilm biofertilizer application rapidly increases soil quality and grain yield in large scale conventional rice cultivation. *Journal of Plant Nutrition*, **46**(7), 1220-1230.

Rennie, R. J. (1981). A single medium for the isolation of acetylene-reducing (dinitrogen-fixing) bacteria from soils. *Canadian Journal of Microbiology*, **27**(1), 8-14.

Sahu, P. K., Tilgam, J., Mishra, S., Hamid, S., Gupta, A., Jayalakshmi, K., Verma, S. K. and Kharwar, R. N. (2022). Surface sterilization for isolation of endophytes. *Journal of Basic Microbiology*, **62**(6), 647-668.

Seneviratne, G., Kecskés, M. L. and Kennedy, I. R. (2008). Biofilmed biofertilisers: novel inoculants for efficient nutrient use in plants. Proceedings of a project workshop, Hanoi, Vietnam, pp. 137.

Seneviratne, G. and Kulasooriya, S. A. (2013). Reinstating soil microbial diversity in agroecosystems. *Agriculture, Ecosystems and Environment*, **164**, 181-182.

Subardja, V. O., Anas, I. and Widyastuti, R. (2016). Utilization of organic fertilizer to increase rice growth and productivity. *Journal of Degraded and Mining Lands Management*, **3**, 543-549.

Wang, L., Zhang, H., Wang, J., Wang, J. and Zhang, Y. (2022). Long-term fertilization with high nitrogen rates decreased diversity and stability of diazotroph communities in soils of sweet potato. *Applied Soil Ecology*, **170**, 104266.

Weerasinghe, D. (2023). Report on policy recommendations for addressing issues in the fertilizer application program for agricultural crops in Sri Lanka, NIFS, Sri Lanka.

Zuberer, D. A. (1994). Methods of Soil Analysis Part 2, In: Weave, R.W., Angle, S., Bottomley, P., Bezdicek, D., Smith, S., Tabatabai, A., and Wollum, A. (Eds.), Soil Science Society of America, Inc., pp 120-142.